CHAPTER IV
RESULT AND DISCUSSION

4.1 Effect of Supplementation (form) either Powdered or Encapsulated Probiotic on Small Intestinal Morphometric

The effect of the use of either powdered or encapsulated probiotic in the villus height (μm), villus width (μm) and crypt depth (μm) showed in Table 6. Based on the results of this research, it can be seen that the administration of probiotic either powdered or encapsulated has no significant effect ($p > 0.05$) on the morphometry of the small intestine (villus height, villus width and the crypt depth). This is appropriate because either powdered or encapsulated probiotic has the same ability to repair the local duck's gut microvilli. This research was supported by Matur and Evren (2012).which reported that growth of microvilli affacted by several factors such as age, sex and type of animal.

Table 6. Effect of supplementation (form) either powdered or encapsulated probiotic on small intestinal morphometric

Treatment	Ileum Morphometric (μm)		
	Villus Height	Villus Width	Crypt Depth
T1	497.33±31.7	119.41±4.1	74.72±5.4
T2	514.52±19.2	122.11±5.8	76.30±3.2
p-value	ns	ns	ns

Note: ns means no signifficant effect

4.1.1 Effect of Supplementation (form) either Powdered or Encapsulated Probiotic on Villus Height

The supplementation of form either powdered or encapsulated probiotic on the villus height showed in Table 6 above. Based on these results, it can be seen that there was no significant effect ($p > 0.05$) between T1 (497.33 ± 31.7 μm) with T2 (514.52 ± 19.2 μm). This is thought to be due to the influence of outside environment variables, such as feed nutrients, environmental conditions of the cage, humidity, temperature and natural factors such as weather and seasons. A study reported by Maiorka *et al.,* (2016) said that difference villus height due to age phase, it also was reported that the height age villi 60 wks longer than the age of 30 wks. The other research resulted from Applegate (1999) reported that the difference between a finisher stage turkey and a starter have different results.

The feed supplemented by probiotic form either powdered or encapsulated to exclude the level of administration, because both have the ability to fix the villus height of local ducks. According to Pelicano *et al.,* (2005) the use of powdered probiotic form which consisted of only *Bacillus subtilis* added at 150 g / ton diet, from 1 to 21 days old; and Probiotics based on *Lactobacillus acidophilus* and *casei, Streptococcus lactis* and *faecium, Bifidobacterium bifidum* and *Aspergillus oryzae* at 1 kg / ton diet, from 1 to 21 days old, equally has significant ($p < 0.05$), villus height from this study was 949 μm, compared with the control and use only prebiotics consisting of Mannan Oligosachharide (MOS) was 898 μm. Its confirmed that either powdered or encapsulated probiotic has an activity to improve the villus height.

The form of encapsulated probiotics is also thought to have the same ability compared to the use of powdered probiotic. The use of encapsulated probiotic has no significant effect ($p < 0.05$) compared to powdered probiotic. This is supposed because at the time of feeding process, occur releasing encapsulant first before entering to target organ. A study of the butyric acid encapsulated afforded to broilers, had no significant results. This lack of a response to sodium butyrate on over the top digestive tract of the crop and proventriculus (Levy *et al.*, 2015). Leeson *et al.,* (2008) reported that lack of intestinal morphology response in the duodenum and jejunum may not differ in duodenal morphology (height villus and crypt depth).

The villus height is influenced by the type of animal, genetic and also feed consumed during the starter phase. The probiotic form either powdered or encapsulated probiotic showed in Table 6 has no significant effect ($P < 0.05$). Nevertheless, from the data shown, the probiotic-encrusted feed tends to increase compared with the powder form. This tendency is suspected because there is an activity process of encapsulated probiotics that can protect while on going digestion process.

4.1.2 Effect of Supplementation (form) either Powdered or Encapsulated Probiotic on Villus Width

The effects of form either powdered or encapsulated probiotic on villus width showed in Table 6. Based on the result of this research, it can be seen that there is no significant effect ($p > 0.05$) between T1 (119.41 ± 4.1 μm) with T2 (122.11 ± 5.8 μm). This indicates the possibility of probiotic either powdered or encapsulated have the same ability and no significant effect

on the villus width. Several research have shown that the width of the intestinal villi is influenced by several genotype factors and animal phenotype. Such as the age, type, feed and sex of object.

The feed contributes greatly to the success of the farm to achieve optimal production. This is because feed as the main source of nutrients in the process of growth and develope animal. Differences in the composition given a major impact on the end results of breeding. Some of the macro components needed by livestock for their metabolic processes are carbohydrates, proteins, fats, fibers, minerals and amino acids. Incharoen *et al.,* (2010) reported that villi characteristic of duodenum and ileum is smaller than feed containing low-CP (crude protein) treatment. Villi in the duodenum has decreased ($p < 0.05$) between treatments containing low-CP and low-CF (crude fat). Thus, its can be seen that the use of feed greatly affects microvillal small intestine of local duck.

Several studies have reported that the villus width is influenced by external environmental factors, such as temperature, humidity, oxygen levels to heat stress. Sugito *et a.,l* (2007) reported that the heat stress in broiler tends to decrease villi width from 1079,34 ± 191,48 μm to 1014,16 ± 192,58 μm. The heat stress increases the coliform microbial growth curve and injects the anaerobic bacteria. This is related to the formation of secretion of mucin compounds as the body's response to elevated levels of the glucorticoid hormone.

4.1.3 Effect of Supplementation of (form) either Powdered or Encapsulated Probiotic on Crypt Depth

The results of this research on the effect of the supplementation form either powdered or encapsulated

probiotic on the crypt depth of local duck showed in Table 6. The results showed that no signifficant effect ($p > 0.05$) between two treatments T1 (74.72 ± 5.4 μm) and T2 (76.30 ± 3.2 μm). This is presumably because the use of either powdered or encapsulated probiotics equally has the same characteristics affected microvilli, especially at the crypt depth. Another studis said, that the crypt depth is also determined by the type of comodities, age and environmental factors, such as feed, drink, temperature and weather.

The type of animal commodities test have a different character in each crypt depth. Another reason was because the microvilli characters of the local duck are relatively shorter than the broiler, so the crypt adjusts the shape of the morphology of the small intestine. Several other studies have shown that probiotic treatment of several types of animal, has different results. Scharek *et al.,* (2005) reported that there was no significant ($p>0.05$) influence on the crypt depths in the pig jejunum which was supplemented by *Enterococcus faecium* 68. Meanwhile, Awad *et al*., (2009) reported that the crypt depth also had no effect in the duodenum, but decreased the crypt depth of Ileum broiler which was supplemented *Lactobacillus sp*.

In addition, crypt depthh is influenced by feed and the environment consumed. Feed affects the metabolic system at GIT. Increased crypt depth indicates that highly mucosal secretions. Probiotics increase the number of cells in the intestinal mucosa and resulted crypt cell migration to tip of villus. Research study from Canonici *et al.,* (2011) reported that *in vitro* and *in vivo* studies were *Saccharomyces boulardii* assisted accelerated migration of intestinal enterocytes through crypt by activating collagen $\alpha 2\beta 1$ receptor.

4.2 Effect of Supplementation (level) either Powdered or Encapsulated Probiotic on Small Intestinal Morphometric

The effect of supplementation of level either powdered or encapsulated probiotic on the villus height, villus width and crypt depth showed in Table 7. Based on the observations, there were a significant effect ($p < 0.05$) on the height and villus width, but no significant effect ($p > 0.05$) to the crypt depth.

Table 7. Effect of supplementation (level) either powdered or or encapsulated probiotic on small intestinal morphometric

Treat(s)	Lvl	Ileum Morphometric (μm)		
		Villus Height	Villus Width	Crypt Depth
T1	L0	489.7±41.35^{a}	118.71±4.99^{a}	74.01±5.88
	L1	490.52±26.83^{a}	119.1±5.31^{a}	74.65±3.93
	L2	511.78±26.25^{b}	120.428±1.68^{a}	75.52±7.24
p-value		*	*	ns
T2	L0	498.10±32.30^{a}	116.56±4.44^{a}	75.27±3.09
	L1	522.21±12.88^{b}	123.07±4.63^{b}	76.67±4.30
	L2	523.26±9.27^{b}	126.7±3.51^{c}	76.96±2.41
p-value		*	*	ns

Notes: ns means no significant effect; * means different superscript within same collum showed any signifficant effect ($p<0.05$)

4.2.1 Effect of Supplementation (level) either Powdered or Encapsulated Probiotic on Villus Height

The effect of supplementation of level nested on the either probiotic or encapsulated probiotic form at the villus height showed in Table 7. The villus height was reported sequences were (μm): T1L0 = 489.7 ± 41.35; T1L1 = 490.52 ± 26.83; T1L2; 511.78 ± 26.25; T2L0 = 498.1 ± 32.30; T2L1 = 522.21 ± 12.88; T2L2 = 523.25 ± 9.27. Statistically, there was signifficant effect ($p < 0.05$) between these levels. This is assume because the level of feed supplemented contains more probiotics, thus optimizing the growth of the villi.

Based on the result of this research, the influence of L1 nested on T1 has no significant effect ($p > 0.05$), that was 490.52 ± 26.83 μm. This is presumably because the use of 0.2% powdered probiotic has not been able to optimally target organ, other allegations are probiotics which consumed decreased survival rate in some digestive organs in the digestive tract. Hermana *et al.,* (2015), reported that enzyme resistance, antimicrobial compounds, pH and bile salts have an inhibitory effect faceing probiotics. Meanwhile, the L2 nested on T1 has the best result ($p < 0.05$) that was 511.78 ± 26.25 μm. This suggests that probiotic levels in the form of powder improve the height of the small intestine villi. It is also suspected because the probiotic level of 0.4% is able to associate with microvilli, probiotics are able to facing bile salts, pH and enzyme activity. Other studies have suggested that *Saccaromyces cerevisiae* contains mannose-based carbohydrates that can increase the height of the intestinal villi. Harimurti *et al.,* (2009) reported that single strain probiotic supplementation of *Lactobacillus murinus, Streptococcus thermophillus* and *Pediococcus acidilactici* increased the height viili of broiler. The villus

height affects the absorption of nutrients during feeding, the longer and wider the villi, the higher absorption process. Furthermore, microvilli determine the feed conversion rasio of animal, resulting in more efficient production (Umniyati *et al.*, 2015).

Based on the results of the research, the effects of the L1 and L2 nested on T2 had the best results ($p < 0.05$) were 522.21 ± 12.88 μm and 523.26 ± 9.27 μm . This suggests that probiotic levels in the form powder improve the villus height. This is suspected, because of the influence of the level of encapsulated probiotics. Ayama *et al.,* (2014) reports current encapsulation is also used as probiotic and applied on poultry, because the combination of probiotics is able to show can withstand low pH activity, high osmotic pressure and high oxygen levels, as well as acid encountered activity in GIT. Liu *et al.,* (2015) reported that encapsulation of *Lactobacillus* isolates with NaCas can protect bacteria during the heating process.

4.2.2 Effect of Supplementation of (level) either Powdered or Encapsulated Probiotic on Villus Width

The effect of supplementation of level nested on levels either powdered or encapsulated probiotic form on villus width has shown in Table 7. The villus width was reported successively (μm): T1L0 = 118.71 ± 4.99; T1L1 = 119.1 ± 5.31; T1L2; 120.428 ± 1.68; T2L0 = 116.56 ± 4.44; T2L1 = 123.07 ± 4.63; T2L2 = 126.7 ± 3.51. Statistically showed in Table 7, there is a significant effect ($p < 0.05$) between these levels. This is presumably because the level of administration contains more probiotics, thus optimizing the villus width.

Based on the result of the research, it is known that nested level at T1 has no significant effect ($p > 0.05$) to the width of villi. This is presumably because probiotics are more susceptible to deterioration of viabilities prior to entry into intestinal microvilli. In addition, the villus width is also determined by the basal feed content consumed. This is in contrast to research conducted Harimurti and Endang (2009) who reported that probiotic supplementation could increase villi width.

Levels of probiotics (L1 and L2) were 123.07 ± 4.63 and 126.7 ± 3.51 nested on T2 had significant effect ($p < 0.05$) on the villus width. This is thought to be due to the effect of probiotic encapsulation level, which is able to withstand probiotics from the contrast of the activities of chemical compounds in the gastrointestinal tract. Patriana (2012) report that probiotics produce enzymes, butyric acids, propionic acid, lactic acid, and bacteriocin that serves to repair the mucosa and villi of the intestines, digestibility, and absorption of nutrients and also suppress harmful bacteria. Agboola *et al.,* (2015) reported that *Lactobacillus sporogenes* and *Saccaromyces cerevisiae* 0.1% in the diet were reported increase the width broiler villi. Libran *et al* (2017) reported that encapsulation with electrospray was able to maintain *Bifidobacterium* cell viablity of 6 log cfu/g at 23% RH. Other studies had reported, Chaickham *et al* (2017) reported that in vitro encapsulated probiotic *maoluang* juice powder with maltodextrin plus *Tiliacora triandra* gum and or inulin, had the ability to maintain survival rate and cell vaiability at 37^{o}C. The microencapsulation of *S. Boulardii, L. Acidophilus* and *Bifidobacterium bifidum* significantly ($p < 0.05$) was detected larger than other

microcapsules during in vitro experiments (Tontul and Mustafa, 2017).

4.2.3 Effect of Supplementation (level) either Powdered or Encapsulated Probiotic on Crypt Depth

The effect of adding levels nested to either powdered or encapsulated probiotic forms at the crypt depth showed in Table 7. The reported crypt depth (μm) were: T1L0 = 74.01 ± 5.88; T1L1 = 74.65 ± 3.93; T1L2; 75.52 ± 7.24; T2L0 = 75.27 ± 3.09; T2L1 = 76.67 ± 4.30; T2L2 = 76.96 ± 2.41. Statistically, there is no significant effect ($p > 0.05$) between these levels. This is presumably because there are other influences that affect the crypt depth, such as feeding factors consumed or probiotics not reaching target organs.

However, from the results of this study, it was found that there is a tendency to increase the crypt depth. Another research, Dong *et al.*, (2016) reported that microencapsulated *Enterococcus faecalis* supplementation had no significant effect ($p > 0.05$) to broiler crypt depth, which was 80.7 μm compared to control treatment 72.6 μm. Villi and crypt have interrelated connections with each other, because the crypt has located in the basal of microvilli. Samli *et al* (2007) reported that *Enterococcus faecium* supplementated in broilers increased the ratio of villus height to crypt depth. In addition, another allegation said no effect on the crypt, beacuse the presence of disturbances in the gastrointestinal tract. Dong *et al.*, (2016) reported that a disruption of GIT epithelial cells causes microencapsulation of *Enterococcus faecium* does not work on the crypt. Another reason have reported that several aspects such as: increased mRNA expression regulation of MUC2, then induced mucus protein secretion.